Summer 1969

The effect of estradiol on protein and nucleic acids of the liver of Natrix fasciata

Alex Jordan

THE EFFECT OF ESTRADIOL ON PROTEIN AND NUCLEIC ACIDS OF THE LIVER OF *NATRIX FASCIATA*

by

Alexander Walker Jordan III

A thesis submitted to the faculty of the Graduate School of the University of Richmond in partial fulfillment of the requirements for the degree of Master of Arts.

August, 1969

THE EFFECT OF ESTRADIOL ON PROTEIN AND NUCLEIC ACIDS OF THE LIVER OF NATRIX FASCIATA

Approved:

F. B. Leftwich
Committee Chairman

Edward Pipe
Dean of the Graduate School

Examining Committee:

W. M. Seaman

William G. Worcott

Wilton R. Tenney

W. R. Wilcox Jr.

R. D. Decker

M. E. Rice

J. W. Fisher

TABLE OF CONTENTS

ABSTRACT

The effects of estradiol on RNA, DNA, and protein concentrations in the liver of Natrix fasciata (southern banded water snake) were investigated. Nucleic acid and protein concentrations were determined spectrophotometrically and expressed as mg/gm wet weight tissue.

Significant increases in both RNA and protein occured 48 hours after a single injection of estradiol. After 72 hours the mean RNA level in estradiol treated snakes had decreased significantly from the 48 hour level. The mean protein level at 72 hours, although lower than the 48 hour level, did not decrease significantly.

Liver DNA concentrations in experimental and control snakes were the same through 48 hours. At 72 hours DNA levels in estradiol treated snakes were significantly higher than untreated snakes.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. Francis B. Leftwich for his advice and guidance throughout the research and writing of this thesis.

I would also like to thank Dr. Willie M. Reams, Jr. and Dr. Wilton R. Tenney for their constructive criticisms of this thesis, and Dr. William H. Leftwich (Psychology Department, University of Richmond) for aid in the statistical analysis of the data.

INTRODUCTION

During the early phase of the estrus cycle in several species of Natrix (water snake) and Thamnophis (garter snake), there is an increase in liver weight and plasma vitellin (phosphoprotein) concentrations. Shortly before ovulation the liver decreases in weight and plasma vitellin concentrations decline to anestrus levels (Dessauer and Fox, 1956 and 1959). Based on these findings and the fact that plasma vitellin is immunologically similar to yolk vitellin (Roepke, et al., 1936), these workers proposed that the liver is the primary site of yolk protein synthesis.

Dessauer and Fox (1959) also demonstrated that estradiol administration to male T. sauritus would initiate synthesis of plasma vitellin and enlargement of the liver.

Boone (1968) showed that estradiol acts directly on the liver to promote protein synthesis. In this study, plasma protein synthesis was stimulated by administration of estradiol to the isolated perfused liver of Natrix fasciata (southern banded water snake).

Hamilton (1963), suggested that estrogen may induce protein synthesis by altering the cytoplasmic biochemical environment to facilitate protein synthesis by ribosomes, resulting in an increase in protein synthesis without an

appreciable increase in RNA levels. In an alternate theory Gorski and Nelson (1965) stated that estrogen participates in intranuclear reactions, the results of which (synthesis of RNA) regulate protein synthesis.

On the premise that new RNA molecules do precede the estrogen-induced synthesis of protein, Hahn and Gorbman (1967) examined RNA levels in estrogen treated and control Uta stansburiana (side-blotched lizard). They found that the rate of liver RNA synthesis was markedly accelerated in estrogen treated animals, which showed a 200 per cent increase over controls.

The present investigation seeks to determine the action of estrogen on liver protein synthesis in N. fasciata. For this purpose liver protein and RNA levels were determined after estrogen administration and compared to untreated controls. Since estradiol is known to induce hyperplasia (Telfer, 1953), DNA concentrations were also measured to determine if any increase in RNA or protein synthesis was due to mitotic activity of the liver.

METHODS AND MATERIALS

A total of thirty-three adult female N. fasciata (southern banded water snake) were obtained from the Tote-Em-In Zoo, Wilmington, North Carolina in early August, 1968 and May 1969 (Table 1). They were kept in screen covered terraria that contained water and used within a four week period after receipt.

Eleven snakes served as an initial control group. These snakes were untreated and used to determine normal liver concentrations of DNA, RNA, and protein.

A preliminary study determined the time required for the maximum estrogen response. Three snakes received a single subcutaneous injection of 1.0 mg estradiol benzoate (in 1 ml sesame oil) per 100 gm body weight. From previous studies by Dessauer and Fox (1959), Clark (1967), and Boone (1968), it is apparent that a dose range of 20-5000 ug of estradiol will elicit protein synthesis without inhibitory influences.

Liver protein and nucleic acid levels were determined 24, 48, and 72 hours after injection. No change over control values in liver nucleic acids or proteins was observed after 24 hours but a very substantial response occured after 48 hours and persisted to 72 hours. From these results it was decided to eliminate the 24

hour sample and to continue with the 48 and 72 hour studies. Liver nucleic acid and protein levels were determined after 48 hours for five snakes and after 72 hours for the remaining snakes.

A third group of snakes was examined for the effects of sesame oil on liver nucleic acids and proteins. Nine snakes were injected subcutaneously with 1 ml of sesame oil and liver RNA, DNA, and protein levels were determined after 48 hours for four snakes and after 72 hours for five snakes.

Snakes were killed by severing the head from the body. From each animal a portion of the liver was extirpated and placed on foil-covered ice. As much connective tissue as possible was removed from the liver specimen and a known weight (approximately 1 gm) of liver tissue was homogenized with four volumes of ice-cold distilled water in a Potter-Elvehjem glass on glass homogenizer for five minutes. Tissue samples were homogenized under cold conditions to retard cellular deterioration. Proteins and nucleic acids were precipitated with 0.6 N perchloric acid (PCA). Lipids were extracted by three successive washings: 95 per cent ethyl alcohol saturated with sodium acetate; 3:1 ethanol ether; and anhydrous ethyl ether. RNA was hydrolyzed into its monoribonucleotides by incubation at 37°C for one hour with 0.4 N KOH. The RNA was precipitated by centrifugation

at 2500 RPM for 10 minutes leaving DNA and protein in solution (Wannemacher, et al., 1965). A biuret technique (Reinhold, 1953) was used for protein determination. A 0.5 ml aliquot of the supernatant was added to 4 ml of biuret reagent and read spectrophotometrically at 550 mμ. Nucleic acid levels were determined using the spectrophotometric technique of Wannemacher, et al., (1965). The remaining protein was precipitated with 2.0 ml of 1.2 N PCA, centrifuged, and 0.2 ml of supernatant was taken for RNA determination. The aliquot was diluted to 10 ml with distilled water and optical densities were read at 260 mμ. DNA was hydrolyzed into its deoxymono-ribonucleotides by incubation for 45 minutes at 96°C with 3 ml of 0.5 N PCA. After centrifugation the supernatant was collected in a dilution tube, diluted to 20 ml with distilled water, and read at 265 mμ and at 290 mμ. DNA absorbs maximally at 265 mμ but there is some absorption due to protein at this wavelength. The contaminating protein was eliminated by subtracting the 290 mμ reading from the 265 mμ reading. RNA, DNA, and protein levels were expressed as mg per gm wet weight tissue.

A two-factor analysis of variance for unequal samples and the t-test were utilized in the treatment of all data (Winer, 1962). Differences between means

were considered significant at the five per cent level of confidence.

RESULTS

Liver protein levels in estradiol-treated snakes were significantly higher than those in the control groups after 48 and 72 hours (Table 3). No such differences existed between snakes treated with sesame oil and untreated snakes. A decrease in the mean protein level (Table 4) from 48 to 72 hours in the estradiol-treated snakes was not statistically significant but may have biological implications.

The mean liver RNA level exhibited a 100 per cent increase over both control groups 48 hours after estradiol injection and remained significantly higher through 72 hours (Table 5). No such differences existed between snakes treated with sesame oil and untreated snakes. In the estradiol-treated snakes RNA concentration decreased significantly between 48 and 72 hours (Table 6).

The liver DNA concentration of estradiol-treated snakes was not different from untreated or sesame oil treated snakes after 48 hours (Table 7). After 72 hours, DNA levels in the estradiol and sesame oil-treated groups were significantly greater than the DNA concentration in the untreated control group. No significant difference existed between snakes treated with estradiol and snakes treated with sesame oil after 72 hours (Table 7). DNA

levels increased significantly from 48 to 72 hours only for the estradiol-treated snakes (Table 8).

DISCUSSION

Liver protein levels in snakes treated with estradiol increased significantly over control levels after 48 hours (Table 3). This evidence that the liver is a primary site for estrogen-induced protein synthesis is in agreement with other investigations. Wallace and Jared (1968) found elevated serum phosphoprotein concentrations and increased *in vitro* uptake of ^{32}P-NaH_2PO_4 by liver slices from *Xenopus leavis* (South African clawed toad), four hours after treatment with estradiol. Boone (1968), using the isolated perfused liver of *N. fasciata*, demonstrated that livers treated with estradiol exhibited a significantly higher uptake of carbon-14 labeled leucine than did control livers.

After 72 hours the mean liver protein levels in estradiol treated snakes had decreased (Figure 1). This may be due to the new protein being released into the circulation. Wallace and Dumont (1968), using *X. laevis*, followed the incorporation of carbon-14 labeled leucine in various tissues of vitellogenic females. Their data show that there is a rapidly labeled protein fraction in the liver which is mostly gone by about 10 hours, after which the amount of labeled protein in the liver declines at a slow rate. The maximum amount of labeled protein in the serum

appears at 8-9 hours, and the level of labeled protein subsequently declines to comparatively low values. The amount of labeled protein in the ovary slowly increases toward a plateau in a manner that generally corresponds with the loss of labeled protein from the serum. Thus it is apparent that plasma vitellin (phosphoprotein) is produced by the liver under the influence of estrogens and that the ovary, more explicitly the oocyte, is the ultimate reservoir for this component.

That serum protein levels in N. fasciata are also elevated in response to estrogen was demonstrated by Boone (1968), who found the highest level of serum protein 72 hours after estradiol administration. This corresponds to the time, as shown in the present study, when liver protein levels apparently are declining.

Earlier investigations have demonstrated that estradiol administration results in an increased synthesis of RNA. Means and Hamilton (1966) reported a stimulation of nuclear RNA synthesis within 2 minutes after estradiol administration in the ovariectomized rat uterus. This stimulation of RNA synthesis was prior to enhancement of either nuclear or cytoplasmic protein synthesis and leaves little doubt that one of the earliest effects of this hormone occurs at the transcriptional level of the rat uterine cell.

Since liver RNA values in the present investigation were determined at daily rather than hourly intervals it is difficult to determine if RNA synthesis preceded protein synthesis. From Figure 2 it is apparent that the maximum level of RNA in estradiol treated snakes occured around 48 hours and declined significantly after 72 hours. Protein levels (Figure 1), although highest at 48 hours, did not decline significantly after 72 hours which may indicate that peak protein levels occured sometime after 48 hours. Thus the results imply that estradiol-induced liver RNA synthesis is prior to protein synthesis.

The marked increase (100 per cent) of RNA levels over control values in snakes treated with estradiol concur with results of Hahn and Gorbman (1967), who found that liver RNA synthesis in estrogen treated U. stansburiana was 200 per cent greater than RNA synthesis in untreated controls. Noteboom and Gorski (1963) found a significantly higher incorporation of ^{3}H-cytidine into RNA in rat uteri treated with estrogen than untreated uteri. Hamilton, et al., (1968) found incorporation of ^{3}H-uridine in rat uterine cell nuclei 500 per cent higher in animals treated with estradiol than untreated rats.

The mechanism of estradiol-induced RNA synthesis is unknown. Hamilton, et al., (1968) reported that the in vitro RNA polymerase reaction in rat uterus nuclei treated with estradiol was stimulated 50-60 per cent over control

values. Noteboom, et al., (1965) hypothesized that estrogen exerts its primary action on the outer membrane of the cell, resulting in an influx of precursors which would permit increased synthesis of nuclear RNA.

Mean DNA concentrations in estradiol treated snakes were no higher than control levels through 48 hours. A significant increase in DNA levels did occur 72 hours after estrogen administration (Figure 3). This late increase in DNA levels in response to estrogen agrees with studies on estradiol-induced DNA increases in rat uteri by Telfer (1953) and Jervell, et al., (1958).

Since peak RNA and protein levels occured at 48 hours it is apparent that these increases were not influenced by estrogen-induced mitotic activity of the liver.

Surprisingly, DNA levels in snakes treated with sesame oil increased significantly over control levels after 72 hours. Both Telfer (1953), and Jervell, et al., (1958), used sesame oil as a vehicle for estrogen administration. Their results show no response to sesame oil at any time. In light of these and other studies, no biological significance is placed on the increase in DNA levels in snakes treated with sesame oil over DNA levels in untreated snakes.

SUMMARY

1. Mean liver protein levels were significantly higher than control levels 48 and 72 hours after estradiol administration.
2. Mean liver RNA levels were significantly higher than control levels 48 and 72 hours after estradiol administration. The mean RNA value in estrogen treated snakes at 72 hours had decreased significantly from the 48 hour level.
3. Mean liver DNA levels were the same in estradiol treated and untreated snakes after 48 hours. Estrogen treated snakes exhibited a significant increase in DNA levels over controls after 72 hours.

LITERATURE CITED

Boone, W. R. 1968 The effects of estradiol benzoate on protein synthesis by the isolated perfused snake liver. Masters Thesis., University of Richmond.

Clark, N. B. 1967 Influence of estrogens upon serum calcium, phosphate and protein concentrations of fresh-water turtles. Comp. Biochem. Physiol., 20: 823-834.

Dessauer, H. C., W. Fox, and N. C. Gilbert 1956 Plasma calcium, magnesium and protein of viviparous colubrid snakes during estrus cycle. Proc. Soc. Exp. Biol. Med., 92: 299-301.

_____, and W. Fox 1959 Changes in ovarian follicle composition with plasma levels of snakes during estrus. Am. J. Physiol., 197: 360-366.

Gorski, J., and N. J. Nelson 1965 RNA synthesis in the rat uterus and its early response to estrogen. Arch. Biochem. Biophys., 110: 284-290.

Hahn, W. E., and A. Gorbman 1967 Liver RNA synthesis associated with estradiol-induced vitellinogenesis. In: Proc. N. W. Reg. Endocrinol. Soc.

Hamilton, T. H. 1963 Isotopic studies on estrogen-induced accelerations of ribonucleic acid and protein synthesis. Proc. Nat'l. Acad. Sci., 49: 373-379.

_____ 1968 Synthesis of ribonucleic acid during early estrogen action. J. Biol. Chem., 243: 408-417.

Jervell, K. E., C. R. Diniz, and G. C. Mueller 1958 Early effects of estradiol on nucleic acid metabolism in the rat uterus. J. Biol. Chem., 231: 945-959.

Means, A. R., and T. H. Hamilton 1966 Early estrogen action : Concomitant stimulations within two minutes of nuclear RNA synthesis and uptake of RNA precursors by the uterus. Proc. Nat'l. Acad. Sci., 56: 1594-1598.

Noteboom, W. D., and J. Gorski 1963 An early effect of estrogens on protein synthesis. Proc. Nat'l. Acad. Sci., 50: 250-256.

_____, J. Gorski, and J. A. Nicolette 1965 Estrogen control of the synthesis of RNA and protein in the uterus. J. Cell. Comp. Physiol., 66: 91-101.

Reinhold, J. G. 1953 Total protein, albumin and globulin. Standard Methods of Clin. Chem., 1: 88-97.

Roepke, R. R., and L. D. Bushnell 1936 A serological comparison of the phosphoprotein of the serum of the laying hen and the vitellin of the egg yolk. J. Immunol., 30: 109-119.

Telfer, M. A. 1953 Influences of estradiol on nucleic acids, respiratory enzymes, and the distribution of nitrogen in the rat uterus. Arch. Biochem. Biophys., 44: 111-119.

Wallace, R. A., and D. W. Jared 1968 Estrogen induces lipophosphoprotein serum of male Xenopus laevis. Science, 160: 91-92.

_____, and J. N. Dumont 1968 The induced synthesis and transport of yolk proteins and their accumulation by the oocyte in Xenopus laevis. J. Cell. Physiol., 72: 73-102.

Wannemacher, R. W., W. L. Banks, Jr., and W. H. Wunner 1965 Use of a single tissue extract to determine cellular protein and nucleic acid concentrations and rate of amino acid incorporation. Anal. Biochem., 11: 320-326.

Winer, B. J. 1962 Statistical Principals in Experimental Design. McGraw Hill Book Co., Inc., New York.

TABLE 1

Body Weight and Length of *N. fasciata*

WEIGHT	SNOUT/VENT LENGTH	TAIL LENGTH	TOTAL LENGTH
	Untreated		
224.4 gm	693 mm	61 mm	754 mm
211.5	714	221	935
162.2	696	168	864
193.4	652	214	866
417.4	812	36	848
195.4	646	51	697
174.4	720	218	938
188.1	712	226	938
116.0	552	217	769
448.2	836	180	1016
254.9	640	215	855
	Sesame Oil Treated		
208.3	691	260	951
154.5	634	85	719
110.4	657	174	831
116.0	552	217	769
253.7	740	245	985
212.5	702	66	768
175.4	693	211	904
116.6	597	164	761
138.9	601	213	814
	Estradiol Treated		
161.3	630	91	721
157.3	585	200	785
352.0	786	229	1015
232.9	648	205	853
228.3	744	231	975
209.1	636	207	843
189.4	605	152	757
639.0	882	304	1186
253.9	781	213	994
172.8	599	202	801
191.6	683	244	927
171.5	612	216	828
114.3	570	240	810

TABLE 2

Analysis of Variance Summary Table

PROTEIN

Source	df	MS	F
time	1	14.49	0.09
treatment	1	5013.98	30.39**
time X treatment	1	310.75	1.88
error	17	165.01	

RNA

Source	df	MS	F
time	1	31.97	22.84**
treatment	1	139.94	99.96**
time X treatment	1	38.97	27.84**
error	17	1.40	

DNA

Source	df	MS	F
time	1	1.14	8.64**
treatment	1	0.08	0.61
time X treatment	1	0.02	0.15
error	17	0.13	

** Significant at the .01 level of confidence

TABLE 3

The Effect of Estradiol on Liver Protein Concentrations[1]

	time after injection							
	48 hours				72 hours			
Comparison	N	$\bar{X}$	SD	t	N	$\bar{X}$	SD	t
estradiol	6	147.5	13.78	6.36**	6	138.8	10.76	4.77**
untreated	11	114.6	12.72		11	114.6	12.72	
estradiol	6	147.5	13.78	4.63**	6	138.8	10.76	3.51**
sesame oil	4	109.2	8.76		5	114.5	11.53	
sesame oil	4	109.2	8.76	0.72	5	114.5	11.53	0.01
untreated	11	114.5	12.72		11	114.5	12.72	

[1] mg/gm wet weight tissue

** Significant at the .01 level of confidence

TABLE 4

A Comparison of 48 and 72 hour Liver Protein Levels[1] in Snakes Treated with Estradiol or Sesame Oil

	estradiol				sesame oil			
Comparison	N	$\bar{X}$	SD	t	N	$\bar{X}$	SD	t
48 hours	6	147.5	13.78	1.17	4	109.2	8.76	0.62
72 hours	6	138.8	10.76		5	114.5	11.53	

[1]mg/gm wet weight tissue

TABLE 5

The Effect of Estradiol on Liver RNA Concentrations[1]

	time after injection							
	48 hours				72 hours			
Comparison	N	$\bar{X}$	SD	t	N	$\bar{X}$	SD	t
estradiol	6	15.31	1.93	12.95**	6	10.04	0.28	4.16**
untreated	11	7.54	0.49		11	7.54	0.49	
estradiol	6	15.31	1.93	10.24**	6	10.04	0.28	3.70**
sesame oil	4	7.49	0.27		5	7.40	0.31	
sesame oil	4	7.49	0.27	0.08	5	7.40	0.31	0.23
untreated	11	7.54	0.49		11	7.54	0.49	

[1] mg/gm wet weight tissue

** Significant at the .01 level of confidence

TABLE 6

A Comparison of 48 and 72 hour Liver RNA Levels[1] in Snakes Treated with Estradiol or Sesame Oil

	estradiol				sesame oil			
Comparison	N	$\bar{X}$	SD	t	N	$\bar{X}$	SD	t
48 hours	6	15.31	1.93	7.72**	4	7.49	0.27	0.12
72 hours	6	10.04	0.28		5	7.40	0.31	

[1] mg/gm wet weight tissue

** Significant at the .01 level of confidence

TABLE 7

The Effect of Estradiol on Liver DNA Concentrations[1]

	time after injection							
	48 hours				72 hours			
Comparison	N	$\bar{X}$	SD	t	N	$\bar{X}$	SD	t
estradiol	6	2.16	0.20	0.22	6	2.72	0.21	2.86**
untreated	11	2.20	0.32		11	2.20	0.32	
estradiol	6	2.16	0.20	0.30	6	2.72	0.21	0.95
sesame oil	4	2.13	0.08		5	2.51	0.19	
sesame oil	4	2.13	0.08	0.30	5	2.51	0.19	1.76*
untreated	11	2.20	0.32		11	2.20	0.32	

[1] mg/gm wet weight tissue

* Significant at the .05 level of confidence

** Significant at the .01 level of confidence

TABLE 8

A Comparison of 48 and 72 hour Liver DNA Levels[1] in Snakes Treated with Estradiol or Sesame Oil

	estradiol				sesame oil			
Comparison	N	$\bar{X}$	SD	t	N	$\bar{X}$	SD	t
48 hours	6	2.16	0.20	2.54*	4	2.13	0.08	1.56
72 hours	6	2.72	0.21		5	2.51	0.19	

[1]mg/gm wet weight tissue

* Significant at the .05 level of confidence

FIGURE 1

The Effect of Estradiol on Liver Protein Concentrations

All values represent means except for the 24 hour figure which represents one sample.

E = estradiol treated

S = sesame oil treated

C = untreated control

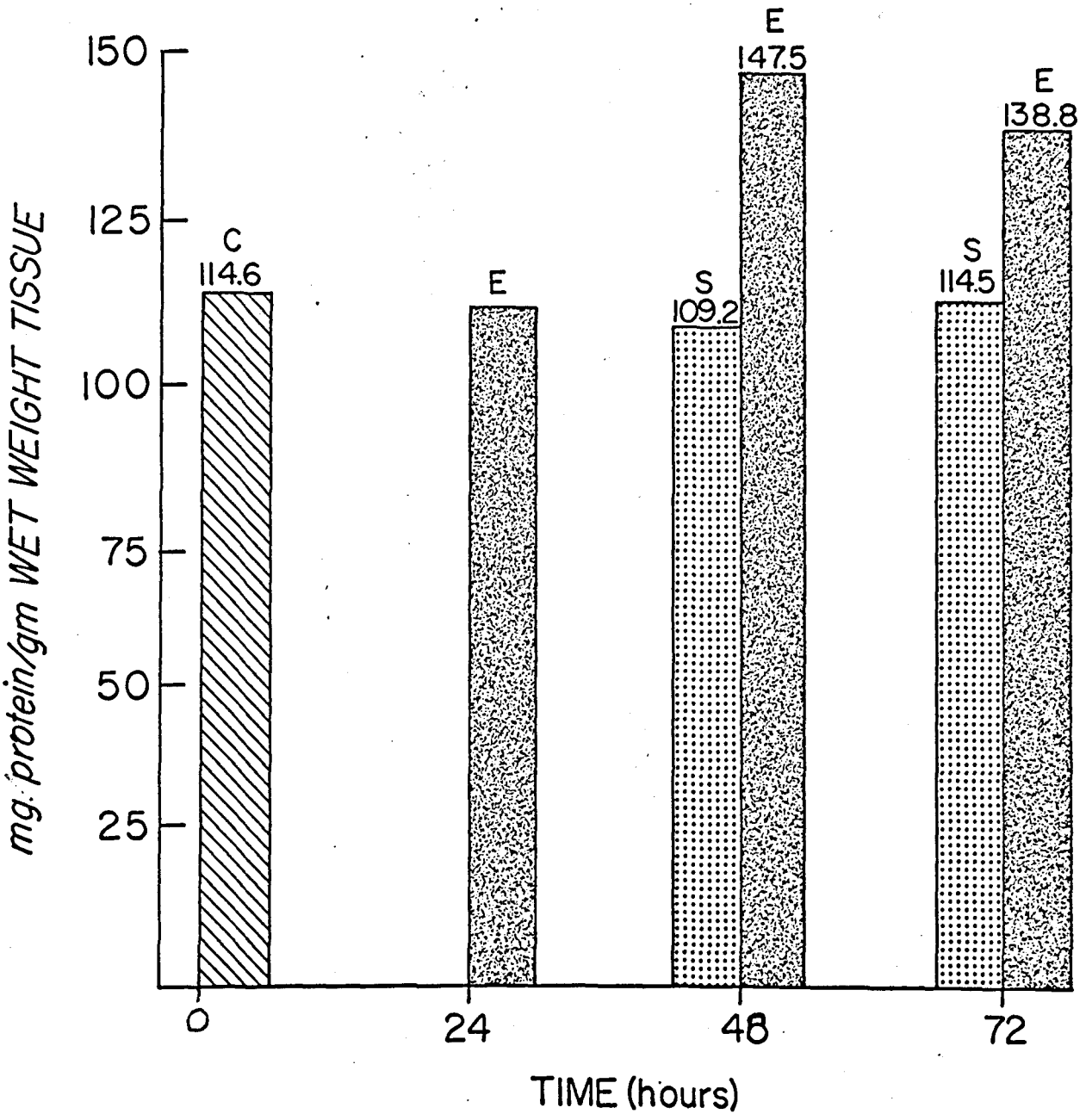

150
125
100
75
50
25
mg. protein/gm WET WEIGHT TISSUE
C
114.6
E
S
109.2
E
147.5
S
114.5
E
138.8
0
24
48
72
TIME (hours)

FIGURE 2

The Effect of Estradiol on Liver RNA Concentrations

All values represent means except for the 24 hour figure which represents one sample.

E = estradiol treated

S = sesame oil treated

C = untreated control

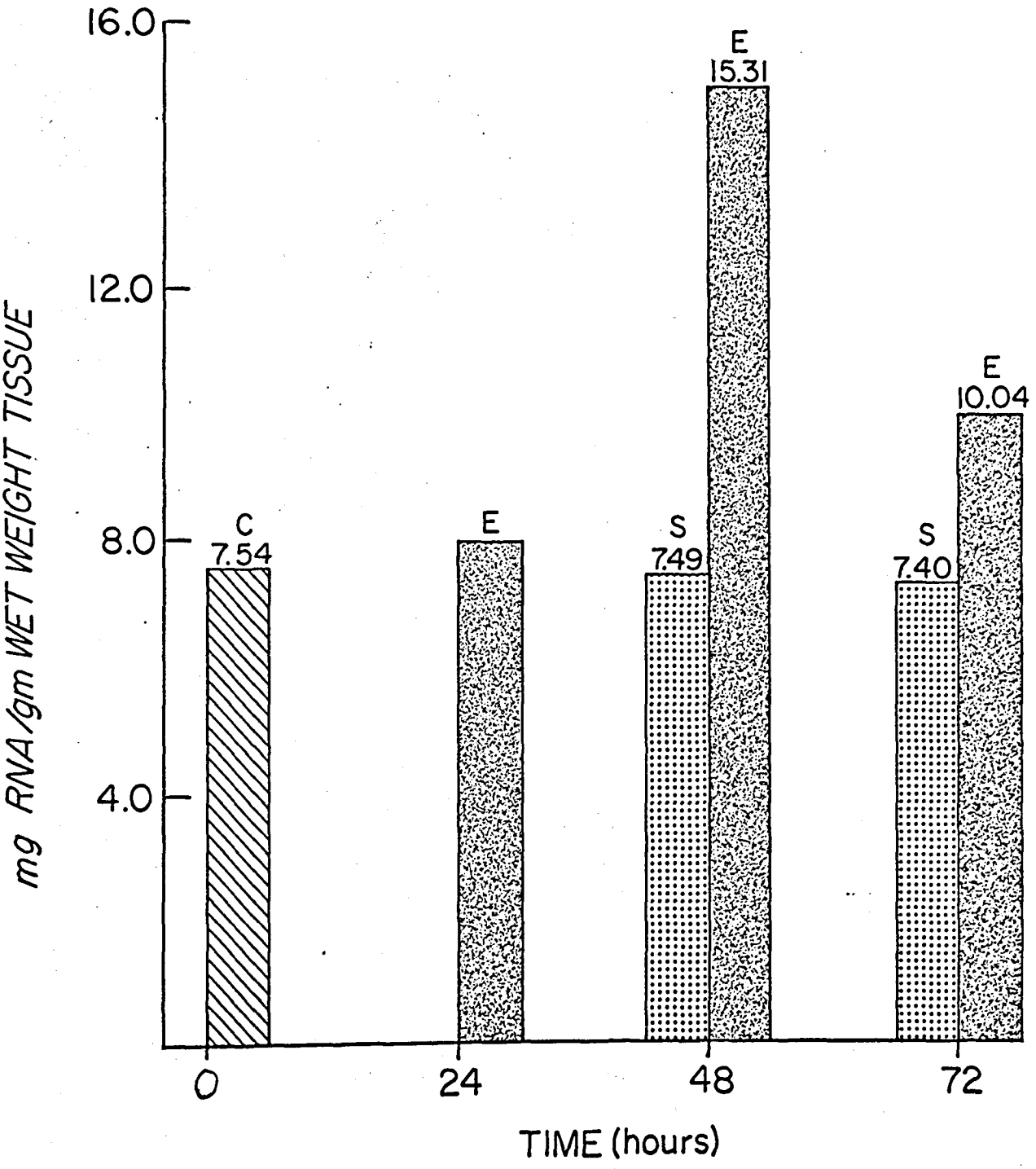

16.0
12.0
8.0
4.0
mg RNA/gm WET WEIGHT TISSUE
C
7.54
E
S
7.49
E
15.31
S
7.40
E
10.04
0
24
48
72
TIME (hours)

FIGURE 3

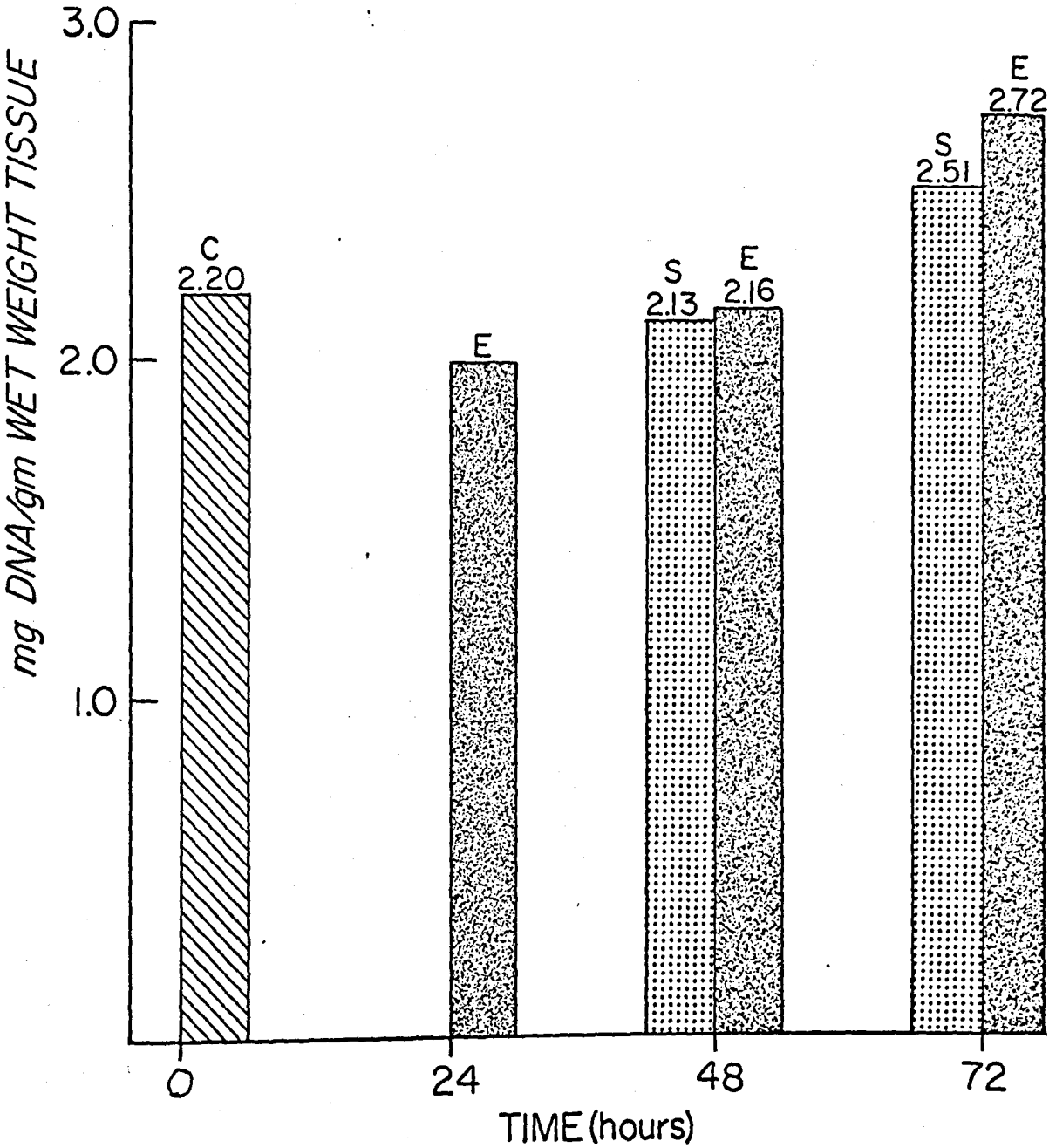
The Effect of Estradiol on Liver DNA Concentrations

All values represent means except for the 24 hour figure which represents one sample.

E = estradiol treated

S = sesame oil treated

C = untreated control


3.0
2.0
1.0
mg DNA/gm WET WEIGHT TISSUE
C
2.20
E
S
2.13
E
2.16
S
2.51
E
2.72
0
24
48
72
TIME (hours)

VITA

Alexander Walker Jordan III was born April 12, 1945, in Richmond, Virginia. He attended Strasburg High School in Strasburg, Virginia and completed his secondary education in June, 1963. After graduation he entered Roanoke College in Salem, Virginia where he majored in Biology. He was graduated in June, 1967 with a B.S. degree, and began graduate study at the University of Richmond in September, 1967. While at the University of Richmond, he was initiated into the Beta Beta Beta Honorary Biological Society. He completed the requirements for the Master of Arts degree in biology in August, 1969. He will enter Rutgers University in September, 1969 to work toward the degree of Doctor of Philosophy.